The striated muscle myosin coiled coil is known to contain regional variations in its functions, such as the polymerization competent light meromyosin (LMM) and the flexible hinge regions. Variations in the structural stability of the coiled coil are an integral part of these functions. To ascertain the variability in stability along the myosin coiled coil, four different regions were analyzed using Free Fall Force Spectroscopy to reversibly pull the two α-helices apart in each location. The targeted regions analyzed were the LMM, and two S2 locations just C-terminal to the S1/S2 hinge defined by site-specific antibodies. Additionally, the S1/S2 hinge was tested by exploiting the rigor bond to impart forces N-terminally to its hinge. As expected, the S1/S2 hinge reversibly unraveled to the greatest extent under less force than the other regions. Regions more C-terminal to the S1/S2 hinge required increasing amounts of force to unravel, and the LMM was the most rigid. A plausible explanation for the variations in stability is that the coiled coil can be pulled apart more readily near its end. In particular, it is observed that regions furthest from a terminus require a high amount of initial force application to separate compared end regions. However, as the strands begin to separate at a site close to the N-terminus, the force-distance curve shapes suggest that the N-terminal part of the coiled coil has completely unraveled and only the C-terminal coiled coil continues to provide resistance. By using the Free Fall Force Spectroscopy, these fine mechanics can be investigated with piconewton to subpiconewton amounts of applied force. It also demonstrates reversibility of the unraveling under repeated cycles of force application on a single molecule. (sponsored by NSF ARRA)

193-Plat
A Novel Mouse Model of Nebulin-Based Nemaline Myopathy
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Nebulin - a giant sarcomeric protein - plays a pivotal role in skeletal muscle contractility by regulating thin filament length and function. Although mutations in the gene encoding nebulin (*NEB*) are a frequent cause of nemaline myopathy (NM), the most common non-dystrophic congenital myopathy, the mechanisms by which mutations in *NEB* cause muscle weakness remain largely unknown. To better understand these mechanisms, we have generated a mouse model in which *NEB* exon 55 is deleted (NEBΔex55), a mutation known to frequently occur in NM patients.
NEBΔex55 mice are born close to Mendelian ratio's, but show growth retardation after birth. Electronmicroscopy shows nemaline rods - a hallmark feature of NM - in muscle fibers from NEBΔex55 mice. Western blotting studies with nebulin-specific antibodies reveal much reduced nebulin levels in muscle from NEBΔex55 mice. Immunofluorescence confocal microscopy studies with tropomodulin antibodies and phalloidin reveal that thin filament length is reduced in muscle fibers from NEBΔex55 mice. In line with reduced thin filament length, the maximal force generating capacity of skinned muscle fibers is reduced in NEBΔex55 mice with a more pronounced reduction at longer sarcomere lengths. Finally, in NEBΔex55 mice the regulation of contraction is impaired, as evidenced by marked changes in cross bridge cycling kinetics and by a reduction of the calcium sensitivity of force generation. This reduced calcium sensitivity was observed only at short sarcomere lengths, suggesting that nebulin might play a role in the length dependence of activation.
In conclusion, we have generated the first nebulin-based NM model. Our data indicate that the phenotype of NEBΔex55 mice closely recapitulates that observed previously by us in patients harboring this particular mutation.

Platform: Chromatin & the Nucleoid

194-Plat
Nucleosome Dynamics Studied by Single-Pair FRET and Computer Simulations
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DNA in nucleosomes is sterically occluded and nucleosomes must open to allow full DNA access. We studied this process by single pair FRET (spFRET), all-atom and coarse-grained molecular dynamics. spFRET shows evidence for a new structural intermediate preceding histone dissociation, in which the (H3-H4)2 tetramer/(H2A-H2B) dimer interface is split open. This is followed by H2A-H2B dimer release from the DNA and, lastly, (H3-H4)2 tetramer removal. This open intermediate state could be demonstrated in Xenopus and yeast nucleosomes; histone variants such as H2A.Z change the mechanism of opening. We estimate that the open state is populated at 0.2 - 3 % under physiological conditions, and could have significant in vivo implications for factor-mediated histone exchange and for DNA accessibility.
To look for subpopulations and changes in FRET distances during the salt-induced transition for the various FRET pairs, we used photon distribution analysis (PDA). We observed two to three populations for all the FRET pairs, their calculated distance corresponding to the values expected from the dye positions in the crystal structure. Histone tail acetylation increased these distances and shifted the midpoint of the opening transition to lower salt concentrations.
The effect of histone tail modification was studied by all-atom MD simulations with 100 ns trajectories of the full nucleosome. We find evidence for an internal allosteric transition at the H2A-H3 interface induced by the removal of either the H3 or the H2A N-terminal tail. DNA dynamics on the histone core are studied by a new coarse-grained model; these simulations show a strong influence of the presence of the histone tails on DNA unwrapping, and an open state that can be stabilized by the displacement of the H3 tail into the gap between the DNA and the histone core.

195-Plat
Kinetics and Thermodynamics of Nucleosome Winding and Unwinding
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Nucleosome post-translational modifications (PTMs), histone variants, and co-factors can activate and repress transcription of the DNA they package. Modified and variant nucleosomes are thought to regulate transcription by two mechanisms acting independently or in concert: ''Tuning'' the DNA binding affinity, or recruiting additional cofactors. To truly understand the regulation of nucleosome packaging processes, it is essential to deconvolute the effects of cofactor recruitment from the effects of changes in binding affinity. To distinguish these two mechanisms, we use single molecule optical trap measurements of nucleosomes under force to measure the kinetics of transitions between nucleosome states. Using a system of known phenotypic response (the sin mutant nucleosomes) we find that nucleosome variations affect the transition rates between states of nucleosome unwinding. From these rates, we are able to determine the free energy difference induced by a nucleosome variation, thus correlating a phenotypic response with rates and energy and giving mechanistic meaning to chromatin ''loosening''. Our measurement of the energy serves as a benchmark against which the kinetics and free energies of nucleosomes containing histones with PTMs, variants, or cofactors may be compared to in future studies.

196-Plat
NAP1-Assisted Nucleosome Assembly on DNA Measured in Real Time by Single-Molecule Magnetic Tweezers
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While many proteins are involved in the assembly and (re)positioning of nucleosomes, the dynamics of protein-assisted nucleosome formation are not well understood. We study nucleosome assembly mediated by the chaperone NAP1 at a single-molecule level in real time with magnetic tweezers. We show that the number of assembled nucleosomes on each of the DNA molecules can be estimated based on the change in supercoiling density and end-to-end length. Furthermore, we demonstrate that this number can be verified by observing the number of disassembly steps when applying high force. With our experimental conditions, we assembled up to ~20 nucleosomes on 8 kb long dsDNA within ~300 seconds. Once the nucleosomes are formed, they are stable, and no disassembly was measured for several hours. Interestingly the association of histones H3 and H4 to the DNA by NAP1 shows a similar DNA compaction compared to nucleosome assembly, but no change in supercoiling density. Histones H2A and H2b with NAP1 do not bind to the DNA.
This data indicate that NAP1-assisted assembly of complete nucleosomes occurs as a two-step process. We suggest that first histones H3 and H4 are associated to the DNA as oligomers, where the DNA is partially wrapped around this substructure, inducing an end-to-end length decrease but no change in linking number. Subsequently, NAP1 brings histones H2A and H2B to the DNA upon which the complete octamer is formed, and the DNA fully wraps around